Quantitative Determination of Sorbic Acid in Cheddar Cheese by Gas-Liquid Chromatography

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A rapid method is described for the extraction and quantitative determination of sorbic acid in Cheddar cheese. Cheddar cheese is impregnated onto a Celite 545 column, extracted with acetonitrile, and analyzed for sorbic acid by gas-liquid chromatography, using a 7.5% ethylene glycol adipate + 2% H_3PO_4 column. Average recovery values for known amounts of sorbic acid were 107%.

Present methods for the determination of sorbic acid in Cheddar cheese are not suitable for performing screening analyses of a large number of samples. Sorbic acid is removed by steam distillation of the sample, followed by solvent extraction and spectrophotometric determination of the sorbic acid in the distillate (1–5). Besides being time-consuming and requiring extensive apparatus, this procedure is not entirely satisfactory, since the spectrophotometric assay gives high blank values and/or is subject to interference by other preservatives which may be present, especially benzoic acid (1, 4, 6).

Attempts have been made to overcome spectrophotometric interference by utilizing differences in absorption maxima at various wavelengths (6–8). Calloway and Schwartzman (9) eliminated benzoic acid interference by measuring the infrared absorption of sorbic acid at 10 nm in chloroform and obtained good recoveries with a variety of samples.

The acetonitrile extraction procedure of Wong and Parks (10), followed by gas-liquid chromatography (GLC) of the extract, provides a simple method for the determination of sorbic acid in Cheddar cheese. Two unique advantages of this method are that the cheese fat is not extracted and that GLC analyses can be performed directly on the extract. GLC analyses of sorbic acid in other foods have been performed (11–13), although to our knowledge none has been adapted to Cheddar cheese. Therefore, it would appear that determination of sorbic acid by GLC would eliminate the disadvantages of high blank values and absorptive interference of other preservatives which may be present, since sorbic and benzoic acids are easily separated by GLC.

METHOD

Apparatus and Reagents

(a) *Diatomaceous earth.*—Celite 545, or equivalent.

(b) *Acetonitrile.*—Redistill before use.

(c) *Sorbic acid.*—Eastman Organic Chemicals, Distillation Products Industries, Rochester, N.Y.

(d) *Sorbic acid standard solution.*—2 $\mu g/\mu l$ in acetone.

Gas Chromatography

Barber-Colman Model 5000 gas chromatograph equipped with hydrogen flame ionization detector was used. GLC parameters: 6′ × 4 mm id stainless steel coiled column containing 7.5% ethylene glycol adipate (EGA) + 2% H_3PO_4 on 90–100 mesh Anakrom ABS (Analabs, Inc., N. Haven, Conn. 06473). Operating conditions: column 165°C and injection port and flame ionization detector 280°C, nitrogen carrier gas 115 ml/min.

Determination

Prepare cheese for extraction according to procedure of Wong and Parks (10). To 20 g finely shredded Cheddar cheese, add 25 g Celite 545 and grind thoroughly. Transfer resulting homogeneous mass to 1.6 × 40 cm column and pack firmly, using packing rod. Rinse mortar with 25 ml acetonitrile; add rinse to column. Let acetonitrile flow into cheese-Celite mixture until level of solvent reaches top of mixture. Add 50 ml acetonitrile to extract sorbic acid from column, eluting into 50 ml Erlenmeyer flask. Let all acetonitrile elute completely from column (total elution time is ca 3 hr, yielding ca 40 ml eluate). Partially remove acetonitrile by evaporation over steam bath, using nitrogen or filtered air. When ca 15 ml acetonitrile remains in receiving flask, quantitatively transfer residual eluate into 25 ml volumetric flask. Rinse receiving flask several times with acetonitrile, transfer all washings into 25 ml volumetric flask, and dilute extract to volume. Inject 2–8 μl extract for GLC analyses.

Obtain standard curves daily in range 4–12 μg, with 6 μg sorbic acid giving 50% full scale deflection. Plot area of sorbic acid peak against μg sorbic acid.

Results and Discussion

Cheddar cheese manufactured by USDA, Dairy Products Laboratory, Beltsville, Maryland 20705, containing no sorbic acid, and commercially available Cheddar cheese, with and without label claim of sorbic acid added as a preservative, were used in this study.

The dose-response curve was determined for sorbic acid over a weight range of 4–12 μg. The area under the GLC peak was calculated by triangulation and a straight line relationship between peak area and mass was obtained for sorbic acid under these conditions.

Figure 1A shows a typical gas chromatogram of the separation of sorbic and benzoic acids; Fig. 1B is a gas chromatogram of Cheddar cheese with sorbic acid and 1C is Cheddar cheese without sorbic acid. GLC analyses of Cheddar cheese prepared at the USDA Dairy Products Laboratory were similar to analyses of commercial brands having no label claim of added sorbic acid. It is apparent that the retention time of benzoic acid is more than twice that of sorbic acid and it does not interfere with the sorbic acid analyses.

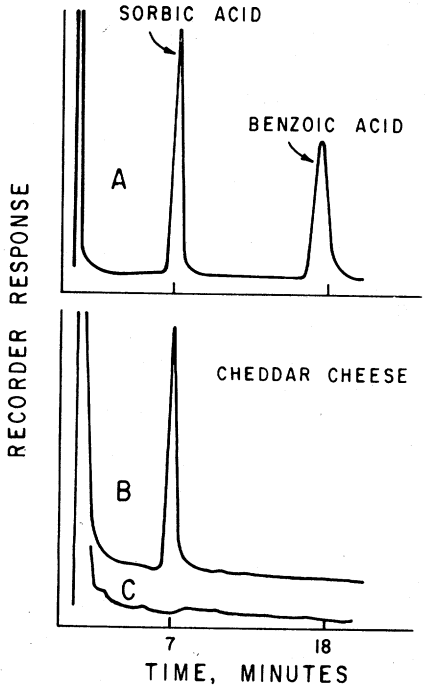

FIG. 1—Gas chromatograms of A, sorbic and benzoic acids; B, Cheddar cheese with sorbic acid; and C, Cheddar cheese without sorbic acid.

The amount of sorbic acid added to Cheddar cheese was equivalent to 0.1 and 0.3%. Twenty g USDA Cheddar cheese was ground thoroughly with Celite 545, and 20 or 60 mg sorbic acid in acetone was added to the homogeneous mass. This mixture was ground thoroughly, packed into the column, and extracted with acetonitrile as described previously. The recoveries fell within a range of 100–112%, based on a total of 20 determinations, with an overall coefficient of variation of 3.35%.

Federal standards for Cheddar cheese, sliced or cut, allow it to contain sorbic acid or its sodium or potassium salt not to exceed 0.3% by weight calculated as sorbic acid (14). Since sorbic acid is preferentially added as a dip for the cut cheese or as a dusting powder to the surface of the cellophane wrapper (15), it would be expected that the distribution of sorbic acid would not be uniform throughout the cheese.

To demonstrate this, a 4 × 14 cm bar of a commercial cheese, having a label claim of added sorbic acid, was sampled at various places. The bar was cut in half and one-half was ground and sampled as representative of the total bar. The other half was cut into slices 1 cm thick, starting from the center of the bar. The first slice was ground and sampled as the center slice. Two samples were obtained from the second slice. One contained the outer 1 cm circumference of the slice and the other a 2 cm square from the center. The values obtained were 0.22, 0.16, 0.26, and 0.095% sorbic acid for total bar, center slice, exterior edge, and center square, respectively. Higher values of sorbic acid were obtained from those sections of the bar having more surface area exposed to the wrapper. Since sorbic acid migrates toward the center from all faces (2), the entire sample must be ground and mixed thoroughly in order to obtain a representative value of sorbic acid. Analyses of several commercially available samples of cheese with a label declaration of sorbic acid showed that sorbic acid values ranged from trace amounts to the legal limit.

The acetonitrile extraction of a cheese-impregnated Celite 545 column provides a fast and efficient method for the isolation of sorbic acid from Cheddar cheese. Subsequent GLC analyses

of the extract provide a rapid, quantitative procedure which is free from interference by benzoic acid and is applicable for routine analyses of a large number of samples. The method is not applicable to cottage cheese, but it should extract other cheeses with a composition similar to Cheddar cheese.

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